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Coincidence of Gaucher's Disease Due to a Private Mutation and Ph′ Positive Chronic Myeloid Leukemia

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We report the case of a 46-year-old female with coexisting type I Gaucher's disease and chronic myeloid leukemia (CML). The diagnosis of Gaucher's disease was made in early childhood by bone marrow biopsy and was recently confirmed by biochemical demonstration of reduced leukocyte β-glucocerebrosidase activity and the presence of Gaucher cells in a bone marrow aspirate. We analyzed the patient's genomic DNA for the underlying glucocerebrosidase mutations and have found homozygosity for a C→T transition in cDNA nucleotide 593 (159 Pro→Leu), presently an undescribed mutation. After initiation of replacement therapy with alglucerase we observed a significant increase of the platelet count in our patient. The diagnosis of CML was based on standard hematological parameters and the detection of the Philadelphia chromosome (Ph). With intermittent treatment with busulfan the patient has remained in chronic phase for nine years. The patient suffered from hepatosplenomegaly and thrombocytopenia, both of which can be caused by Gaucher's disease and CML. The aggravation of skeletal manifestations of Gaucher's disease, which occurred at the time of diagnosis of CML, could be due to increased production of leukocyte-derived glucocerebrosides that were not appropriately degraded because of the genetic β-glucocerebrosidase deficiency. Am. J. Hematol. 59: 87–90, 1998. © 1998 Wiley-Liss, Inc.

Key words: Gaucher's disease; chronic myeloid leukemia; alglucerase; mutation analysis

INTRODUCTION

Gaucher's disease is the most common lysosomal storage disease [1,2]. Three major clinical types (I, II, and III) of the disease are known. Hepatosplenomegaly, pancytopenia, skeletal involvement, and elevated serum angiotensin converting enzyme (ACE) and acid phosphatase levels are typical for all types. Neurologic involvement occurs as a primary disease manifestation only in patients with type II (acute neuronopathic) or III (subacute neuronopathic) disease. The disease results from a genetic deficiency of the lysosomal enzyme β-glucocerebrosidase, causing an abnormal accumulation of glucocerebrosides in macrophages. Glucocerebrosides are naturally occurring glycolipids produced by enzymatic degradation of leukocyte and erythrocyte membranes. At present, more than 70 different mutations in the β-glucocerebrosidase gene that cause this autosomal recessive disorder are known [3,4]. The typical histological feature of Gaucher's disease is the presence of Gaucher cells in liver, spleen, or bone marrow. These cells are lipid-laden macrophages with fine linear cytoplasmic striations. Similar cells are also observed in chronic myeloid leukemia [5–8] where they are called Pseudo-Gaucher cells. When examined by electron microscopy, typical Gaucher cells [9–12] and Pseudo-Gaucher cells in CML both contain cytoplasmatic sacs with inclusions which differ, however [13,14]; in CML, they are linear densities with a fibrillar pattern, whereas in Gaucher disease, they are long and twisted 300 to 400 Å wide tubes.

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Pseudo-Gaucher cells in multiple myeloma [15] contain crystalline structures of various diameters enclosed in large membrane bound vesicles.

CASE HISTORY

Clinical Data

The patient is a non-Jewish 46-year-old female who was born in 1951 in Greece of parents who came from the same village and emigrated to Germany in 1969. Gaucher's disease was first diagnosed by bone marrow aspiration and demonstration of Gaucher cells in 1959 when the patient was eight years old. Two of five siblings were also diagnosed to have this disease. In 1962 and 1971 the two siblings with Gaucher's disease died of hemorrhagic complications. At this time the patient only had occasional epistaxis. No neurologic manifestations were found, indicating type I Gaucher's disease. Between 1971 and 1972, postoperative hemorrhagic diathesis became apparent after appendectomy and an abortion. In 1972 the patient gave birth to a healthy son. By 1973 massive splenomegaly (three kg) had developed so that splenectomy was performed. Skeletal involvement with arthralgia in all extremities started to develop in 1980 and replacement of both hips after osteonecrosis was necessary in 1987 and 1988. During these years an elevation of the white blood cell count was observed for the first time (see below). Allogeneic bone marrow transplantation from her HLA (human leukocyte antigen) compatible sister was discussed and refused by our patient.

Hematological Data

A clinical diagnosis of CML was made early in 1988 after a period of six months with significant weight loss, fatigue, and leukocytosis with a maximum white blood count of 48,000/μl. This diagnosis was confirmed by cytogenetic demonstration of the Philadelphia chromosome (Ph). Additional laboratory findings in early 1988 included the following: A left shift in the myeloid series (myeloblasts, 1%; promyelocytes, 14%; myelocytes, 13%; metamyelocytes, 12%; neutrophils, 46%; basophils, 2%; and eosinophils, 2%), a leukocyte alkaline phosphatase score of four, and an elevation of lactate dehydrogenase (LDH) (646 U/l). Platelet counts (231,000/μl) and hemoglobin (13.3 g/dl) were normal. Therapy of CML was initiated in November 1988 with intermittent administration of busulfan until February 1993. Since the beginning of 1990 clinical symptoms have included chronic fatigue and arthralgia in both wrists and the left knee. Since 1993 the patient has remained in chronic phase without cytoreductive therapy. However, a continuous decline of the platelet count to values below 30,000/μl with an increasing bleeding tendency was noted. A marrow biopsy with an additional electron microscopic analysis was performed (Fig. 1).

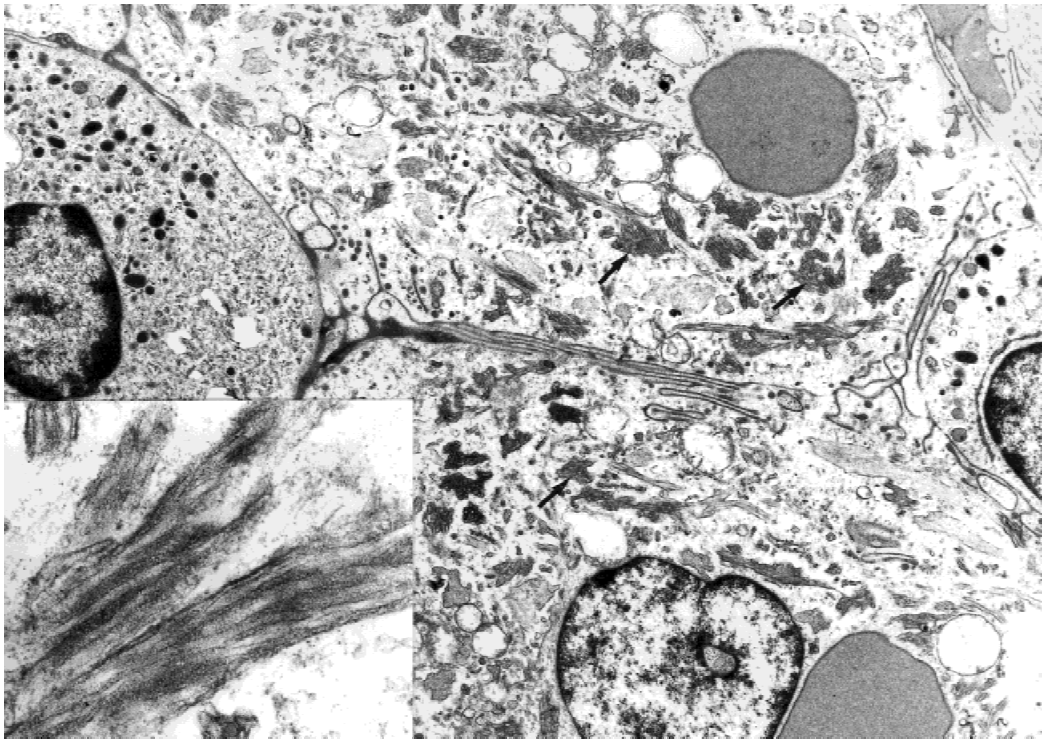

Fig. 1. Ultrastructure of Gaucher cells of our patient showing typical tubular formations (20–40 nm) spiraling in right-handed screw sense (see arrows) along the tubule. (Magnification, ×10,000; inset, ×76,000.)

β-Glucocerebrosidase Enzyme Activity and Gene Mutation Analysis

The residual β-glucocerebrosidase activity was determined by using glucosyl (^{3}H-) ceramide as a substrate [16]. The method was modified to a 1.8 μM concentration of this substrate in 0.2 ml acetate buffer, pH 5.5 with 3.5×10^6 white blood cells. After 180 min incubation at 37°C, 1.94% of the substrate were degraded by the patient's leukocytes (normal mean, 11%; range, 5–19%). The patient's DNA was screened for eight common mutations (1226G, 1448C, IVS2+1, 84GG, 1297T, 1504T, 1604A, and 1342C) that account for more than 70% of all mutations in non-Jewish patients [17]. When none of these mutations were found, direct sequence analysis of purified polymerase chain reaction (PCR) fragments along the entire length of the gene was performed using fluorescence-labeled primer dideoxynucleotide chain termination protocols and the 373A sequencer (Applied Biosystems, Perkin Elmer, Foster City, CA). For PCR amplification of 0.25–0.5 μg of genomic DNA, oligonucleotide primers were chosen to prevent amplification of the pseudogene. The PCR was performed in a Perkin Elmer GeneAmp PCR System 9600. DNA was initially denatured at 98°C for four min. The conditions of PCR were as follows: 94°C for 30 sec; 58°C for 30 sec; and 72°C for 30 sec. Final extension was for seven minutes. A homozygous novel mutation (nucleotide 593 C→T leading to amino acid 159 Pro→Leu) was identified in the β-glucocerebrosidase gene of our patient.

The sequences of the primers used to amplify the fragment in which the mutation was found were as follows: 5′-GCAGAGTCCCATACTCTCCT-3′ and 5′-TAGTTGGGTAGAGAAATCG-3′. Thirty cycles were used to provide a product that was 1,465 bp in length. Sequencing of this fragment was performed using the oligomer 5′-GTGTTCCAACTCTGGGTGCT-3′.

Enzyme Replacement Therapy With Alglucerase

Due to the thrombocytopenia with a bleeding tendency, we initiated therapy with alglucerase in August 1995, using the protocol proposed by Hollack et al. [18]. Prior to therapy with 3 × 100 U alglucerase/week, the platelet count was 30,000/μl; hemoglobin, 12.8 g/dl; leukocytes, 6,200/μl; ACE, 165.0 U/l; acid phosphatase, 30.4 U/l, and the body weight was 70 kg. After three months of therapy a significant increase of the platelet count to 70,000/μl as well as a decrease of ACE to 120.0 U/l and acid phosphatase to 19.4 U/l were noted. The liver volume measured by quantitative computer assisted tomography was unchanged at 1,925 ml. During this period clinical symptoms such as fatigue and headache became less pronounced so that the patient requested discontinuation of treatment in December 1995. In February 1997 the platelet count had again fallen to 24,000/μl with increasing hemorrhagic diathesis and alglucerase therapy was resumed (2 × 150 U per week). In June 1997 the platelet count had increased to 94,000/μl.

DISCUSSION

Patients with Gaucher's disease who also have lymphoproliferative diseases such as chronic lymphocytic leukemia, multiple myeloma and Hodgkin-lymphomas have been reported [19–21]. However, the prevalence of neoplastic disorders in patients with Gaucher's disease has not been determined. To the best of our knowledge, coexisting Gaucher's disease and CML has only been reported once in the world literature [22]. In contrast to our report, the patient presented by these authors was diagnosed to have Gaucher's disease after the manifestation of the CML, indicating that the Gaucher's disease was less severe. Their patient received treatment with busulfan upon diagnosis and entered a fatal blast crisis after 14 months, whereas our patient is still in chronic phase nine years after initial diagnosis. Mutation analysis in our patient has revealed a novel mutation, i.e., a C→T transition in cDNA nucleotide 593. This produces a replacement of proline by leucine in amino acid 159 of the processed enzyme. A mutation analysis of 10 Greek patients with type I disease has led to the identification of three mutations (1226G, 1448T, 1504C) that comprised 80% of the investigated alleles [23]. In another study, the 1342C mutation was found in a Greek family [24]. Interestingly, our patient is homozygous for the private mutation that we have found. This presence of two identical mutations may reflect the fact that both of her parents come from the same village, i.e., that their families may be related to each other.

As lipid-laden macrophages in our patient were present long before the manifestation of the CML, we consider these cells to be true storage cells. This is confirmed by electron microscopic analysis which shows tubular formations characteristic for Gaucher cells. Because therapy with low-dose alglucerase resulted in a significant increase of the platelet count, the thrombocytopenia has to be attributed to the Gaucher's disease, although a contribution of the CML cannot be completely ruled out. Interestingly, in our patient, a coincidence occurred between the increase in skeletal manifestations of Gaucher's disease and the clinical manifestation of the CML in late 1987 and early 1988. During that time the patient required replacement of both hip joints. At the time of surgery our patient already had a six-month history of weight loss and leukocytosis leading to the diagnosis of CML in February 1988. The presence of the so-called pseudo-Gaucher cells in hematological malignancies such as CML has been known since the initial description by Albrecht thirty years ago [5]. These cells also contain glucocerebrosides as do true Gaucher cells but differ in

their electron microscopic appearance (see above). Since the leukocyte membrane is the major source of β-glucocerebrosides, the underlying mechanism for the development of these cells is an overproduction of β-glucocerebrosides, which is caused by the leukocytosis. In our patient, the coincidence of both an acquired overproduction of β-glucocerebrosides by CML and an inborn reduced degradation by Gaucher's disease may have aggravated the bone involvement.

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